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RESEARCH ARTICLE

Phytochemical and Biological Screening of *Lantana camara* Linn. Leaves Extract

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Abstract

The current study evaluated the phytochemical and biological screening of L*antana camara* (Verbenaceae). It is a highly invasive ornamental garden plant species, native to tropical and sub tropical America. Leaves of *L. camara* were extracted successively by petroleum ether and methanol by continuous hot percolation. The phytochemical screening was carried out by colour reaction with different reagents. Well diffusion on Mueller Hinton agar was used for evaluating antimicrobial activity. The LD_{50} value was determined by acute toxicity studies. The analgesic activity was carried out by tail immersion method, antimotility activity was carried out by charcoal movement test and the antidiabetic activity was carried out by oral glucose tolerance test. The extractive value of methanolic and petroleum ether was found to be 10.11% and 3.11% respectively. The preliminary phytochemical screening showed the positive reaction test for glycoside, tannin, saponin, steroid, flavonoid, carbohydrates, diterpine, triterpine.The extract showed significant antimicrobial activity against *S. aureus* ($p<0.05$) and didn't show any activity against *E. coli*. The LD_{50} value was found above 2000mg/kg. The analgesic, antimotility and antidiabetic activity of plant extract showed the significant results in dose dependent manner ($p<0.05$). The study revealed that the plant possessed antibacterial, analgesic, antimotility and antidiabetic activity. Further studies are needed to standardise the extract and evaluation of safety profile in long-term toxicity studies are recommended.

Key words: *Lantana camara*, LD_{50}, analgesic, antimotility, antidiabetic and antimicrobial activity.

Introduction

Plants have been one of the important sources of medicine even since the beginning of human civilization[1]. A large proportion of the population of developing countries uses traditional medicines, either as a result of the high cost of Western pharmaceuticals and health care, or because the traditional medicines are more acceptable from a cultural and spiritual perspective [2-5].

Total 60% of population of the world and 80% of the population in developing countries like Nepal rely on ethno-medicine for their primary health care needs which is mainly derived from plant and many pharmacopoeias still contain at least 25% drugs derived from plants [6-9]. Hence the approach to new drugs through natural products has proved to be the single most successful strategy for the discovery of new drugs. A total of 1950 species of medicinal plant are used in Nepal and out of which 1906 species are identified under vascular group comprising 1614 native, 192 introduced/ cultivated, 100 naturalized taxa [10-13]. According to WHO, the country ranks 27th on the global scale and 11th on the continental scale in number of flowering plants which is 2.5 % of global flora [14-16]. In an assessment undertaken by IUCN in Nepal during 2002-2003, 21 naturalized plant species have been reported as invasive in Nepal and *Lantana camera* is one of them [17-19].

L. camara is a highly invasive ornamental flowering plant belonging to family Verbenaceae [20]. It is native to sub tropical and tropical America and commonly known as wild or red sage. It have been used by folk healers in Asia and South America to treat various dermatological and gastrointestinal diseases, malaria and tumors [21-22]. It is a perennial, summer-growing, erect shrub, growing up to four meters high and often forming dense thickets. Plant produces strong aromatic order. It grows up to 2000 m in tropical, sub tropical and temperate region. Root system is very strong and gives out repeated shoot after repeated cutting [23]. The plant is said to possess principle constituents like essential oil, terpenoids, flavonoids, alkaloids, glycosides and steroids [24-27]. The major constituents in the essential oil of leaves are Germacrene-D (20.5%), γ-Elemene (10.3%), β-Caryophyllene (9.4%), β-Elemene (7.3%), α -Copaene (5.0%) and α-Cadinene (3.3%) [28-29]. Mono and sesquiterpines such as Curcumenes, Bisabolene and Safrole are also present in oils of leaves and flowers [29]. Triterpines such as Lantadenes A, B (toxic), C and D, Lantanolic acid are found leaves and stems. Multiple methoxylated derivatives of Quercetin are also reported in leaves [30].

The nature has always been a reservoir of potentially useful bioactive compounds which provide newer leads and pathways for modern drug discovery [31]. The knowledge of traditional medicine and medicinal plants and their study of scientific chemical properties may lead to the discovery of newer and cheaper drugs [32]. Besides, plants are with traditional uses which have treated many diseases since time immemorial, and research on validation of ethno medicine is the topic of interest for now [33]. This study is designed to analyze possible antimicrobial, analgesic, anti motility, hypoglycemic action of the plant. This can ultimately encourage further research work on the plant.

Materials and methods

Study design and site: This is an Experimental and exploratory study design. The research was carried out in the Chemistry and Pharmacology laboratory of the Pharmacy department of Maharajgunj Medical Campus as well as the Pharmacology Laboratory of Natural Products Research Laboratory Thapathali, Kathmandu. The antibacterial activity was carried out in Microbiology laboratory of Maharajgunj Medical Campus, Maharajgunj, Kathmandu.

Plant collection and Identification: Wild collection of the plant was done from Tokha-7, Kathmandu in the month of August and identified as *Lantana camara Lin*n in the National Herbarium and Plant Laboratory, Godavari, Lalitpur.

Plant processing and extraction: The leaves of *L. camara* were dried at room temperature for two weeks. The dried sample was crushed into powder by grinder and passed through 60 Sieve. The dried powder was kept in airtight container protected from direct sunlight. About 60 gm of powder material was placed in a thimble and extracted successively with 400 ml of Petroleum ether and Methanol in Soxhlet apparatus at 60 °C. The extraction was continued till the solvent becomes colorless. The extract was evaporated to dryness using rotary vacuum evaporator. The crude extract was weighed and percentage yield was calculated, and these crude extracts were stored in air tight container (inside refrigerator) for further studies.

Phytochemical Screening: The phytochemical screening was done to characterize the chemical constituents present in different extracts of *L. camara* by their color reactions with different reagents. Each extract was subjected for alkaloids, glycosides, tannins, diterpine, flavonoids, carbohydrates, protein, steroids, saponins using the standard procedure.

Antimicrobial screening: Well diffusion method was used to determine antimicrobial activity with slight modification [34-35]. Isolated strains of *Staphylococcus aureus and Escherichia coli* were used for screening of anti-microbial activity of plant extracts. Microorganisms and antibiotics disc of reference standard (Gentamicin) were obtained from the Microbiology laboratory of Tribhuvan University Teaching Hospital (TUTH), Kathmandu. Anti-microbial screening was performed in the methanolic

extract of *L. camara* (MELC). The extract solutions were prepared by dissolving in 5% DMSO and were prepared of different concentration.

Acute oral toxicity: The acute toxicity study of MELC was carried out as per OECD guideline. 423 (Acute Toxic Class method) [36]. It involves the identification of dose level that causes mortality. MELC at high dose 2000 mg/kg was given orally to overnight fast five mice and observed at regular interval for 7 days. Then the doses were selected for further study.

Analgesic activity: The animal model employed for screening of analgesic activity in this study was Tail immersion method [37-38], which includes pain state models using thermal stimuli. For carrying out the Tail immersion test Mice of 20-35 gm were divided into five groups of four mice each. The first group was given 2% Tween 80 at 10 ml/kg as control. The second group was given aspirin 300 mg/kg orally made in distilled water as standard. The third, fourth and fifth group were given MELC in three different concentration 400 mg/kg, 600 mg/kg, 800 mg/kg made in 2% Tween 80 v/v orally at 10 ml/kg body weight. About 5 cm from the distal end of the tail of each rat was immersed in warm water maintained at 50˚C. The reaction time is the time taken by the mice to deflect their tail due to pain. The reaction time was taken as average of 3 reading. The reaction time was recorded before 0 min and at 30, 60, 120 min after the administration of treatment. Cut of time of 15 sec was observed to prevent the any tissue damage to the animal. The maximum possible analgesia (MPA) was calculated as below.

$$\text{MPA} = \frac{time\ of\ treatment\ reading - time\ of\ control}{15 - time\ of\ control} * 100$$

Antimotility test: The Charcoal meal test was used to evaluate the efficacy of a compound to inhibit gastrointestinal motility in small intestine [39-41]. This test is based on the intestinal transport of charcoal meal along the small intestine. For carrying out the antimotility test 20-30 mg mice were fasted for 24 hours but allowed free access to water. They are randomized into five groups of four animals each. Each animal of group first were administered control vehicle (2% Tween 80 orally) at 10 ml/kg. The animals of second group were administered loperamide 4 mg/kg orally as standard. Group third, fourth, and fifth animals were administered 400 mg/kg, 600 mg/kg, 800 mg/kg MELC made in 2% Tween 80 solution by oral route respectively. After 15 min, each mouse were administered 0.3 ml charcoal meal (10% activated charcoal suspension in 10 % Tragacanth gum) orally with the aid of feeding needle. These mice were killed after 30 minutes by inhalation of chloroform. The abdomen opened, the intestines were quickly isolated and the small intestine from pylorus to ceacum was cut by scissor and its length was measured. The distance traversed by charcoal meal from pylorus to caecum was expressed as a percentage of the total length of the small intestine.

$$\% \text{ inhibition} = \frac{\text{Distan}}{\text{total}} * 100$$

Where, Dc: Mean distance travelled by the charcoal in the control group and

DT: Mean distance travelled by the charcoal in the test group

$$\text{Intestinal propulsion } \% = \frac{\text{Distance travelled by charcoal}}{\text{total distance of intestine}} * 100$$

Antidiabetic activity: It was evaluated using oral glucose tolerance test [42-43]. This method measures body's ability to use or remove excess sugar from blood and also used to screen hypoglycemic properties of products/extracts. In this method, rats about 148-158 g were fasted for 16-18 hours but allowed free access to water. The fasted rats were divided into 4 groups, 4 rats each. Initial blood glucose level of each rats were measured by tail- tip method using glucometer. Group I were given Glibenclamide 5mg/kg orally made in distilled water as standard. Group II were given MELC orally at 400 mg/kg dissolved in 2% tween 80 v/v. Group III were given methanolic extract orally at 800 mg/kg dissolved in 2% Tween 80v/v. Group IV were given 2% Tween 80 v/v orally as control. After 30 minutes of the extract and drug treatment the rats were loaded with 3 g/kg body weight glucose solution. The blood glucose levels of rats were then measured at 30 min, 60 min, 90 min after the glucose load. The percentage, variation of glycemia was calculated as a function of time according to the following formula:

$$\% \text{ Induced glycaemia} = \frac{(Gx) - (Go)}{(Go)} * 100$$

Where Go is initial blood glucose level before glucose load and Gx is blood glucose level at 30 min, 60 min and 90 min. The tolerance of rat to glucose gives indication of presence of hypoglycemic activity.

Results

Extractive value: The total yield percentage of petroleum ether and methanol was calculated as shown in table 1.

Table 1. ***Extractive value of methanolic and petroleum ether of L. camara leaf***

Extract	Yield percentage (%)
Methanol	10.11
Petroleum ether	3.12

Phytochemical Screening: Phytochemical screening of the methanolic and petroleum ether extract of leaves of the plant showed various group of active constituents as shown in the table 2.

Table 2: ***Phytochemical screening Extract of Lantana camara leaves***

S.N	Test	Methanolic extract	Petroleum extract
1	Tannins	+	-
2	Diterpine	+	+
3	Triterpine (salkowski test)	+	-
4	Flavonoids(Shinoda test)	+	-
5	Carbohydrate		
	Molish Test	+	+
	Felling Test	+	+
6	Protein		
	Xanthoprotein	-	-
7	Steroid	+	+
8	Saponin(foam test)	+	+
9	Alkaloids		
	Hagers, Wagners, Mayer's	-	-
10	Glycosides		
	Brontrager's Test, legal test	+	-

Note :- "-" Absent,"+" Present

Antimicrobial activity Analysis: The bacterial strains were obtained from the Microbiology laboratory of TUTH, Kathmandu. The effectiveness of plant extract was tested against *E. coli* and *S. aureus* (table 3). The diameter of borer was 5mm.

Table 3: ***Antimicrobial activity of MELC against gram positive and gram negative organism.***

Sample	Concen-tration	Mean zone of inhibition(mm) ±S.E. Micro-organism used	
		S. aureus	*E. coli*
Control	25% DMSO	-	-
Gentamicin	10 mcg	21.67±0.882*	32.33±0.882
Extract	150 mg/ml	11± 2.082*	-
Extract	200 mg/ml	12.33±1.333*	-
Extract	400 mg/	13.67± 0.667*	-

*p<0.05

The results showed extracts were active against *S. aureus*. The zone of inhibition of 150 mg/ml, 200 mg/ml, and 400 mg/ml of **MELC** was found to be 11± 2.082 mm, 12.33±1.333 mm and 13.67± 0.667 mm respectively against the *S. aureus* where as the standard Gentamicin (10mcg) was found to be 21.67±0.882 mm (p<0.05). The plant extract didn't show any zone of inhibition against *E. coli.*

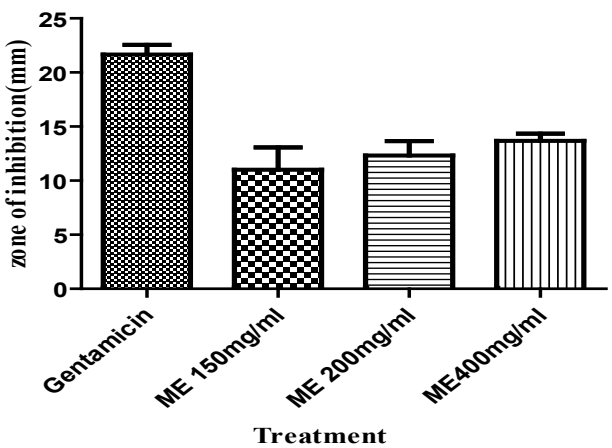

Figure 1: ***Antimicrobial action of Gentamicin and MELC against S. aureus***

Acute oral toxicity: In the acute oral toxicity the MELC treated mice at a higher dose of 2000 mg/kg did not exhibit any sign of toxicity and difference in behavior at any time of observation. There was no mortality in the above mentioned dose at the end of

7 days of observation. Therefore the lethal dose LD_{50} was considered above 2000 mg/kg.

Analgesic activity: The analgesic activity of MELC was determined by tail immersion method. The mice treated with control (2% Tween 80) didn't show any significant difference in reaction time throughout 120 minute. The highest reaction time for extract 800 mg/kg was 6.83±0.03 sec and aspirin 300 mg/kg was 7.49±0.04 sec at 60 minute after which the activity begins to decrease. The analgesic effect of aspirin and extract was evident within 30 minute and reach at peak value at 60 min. The maximum possible analgesia at 60 min by aspirin 300 mg/kg, extract 800 mg/kg, extract 600 mg/kg and extract 400 mg/kg was found to be 40.72%, 35.5%, 28.07% and 19.13%. There was no significant difference of reaction time between the extract 800 mg/kg and aspirin 300 mg/kg at 30 min and 120 min($p>0.05$).

Values are mean ± SEM of 4 mice. The statistical significance was tested by using ANOVA, post hoc Tukey method. The mean difference between the control, standard and different doses of extract was found to be significant ($p<0.01$). Also the significant dose dependent activity of the plant extract has been found.

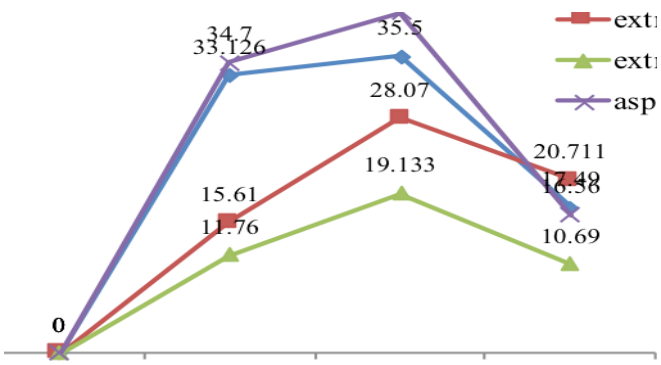

Figure 2: ***Analgesic effect of Aspirin and different concentrations of MELC***

Antimotility activity: The antimotility activity of MELC was carried out by the charcoal movement test and the MELC 400mg/kg, 600mg/kg and 800mg/kg showed significant antimotility activity versus control(2%v/v Tween 80) and standard drug (Loperamide 4mg/kg). There was significant difference in antimotility activity between different concentration of MELC i.e. they exhibit dose dependent antimotility effect. The intestinal transit at a dose of 400mg/kg, 600mg/kg and 800mg/kg were 58.26±0.615%, 47.91±0.620%, 27.83±1.09% whereas of control and loperamide 4mg/kg were 92.35±0.563% and 33.34±1.01% respectively.

Table 5: ***Antimotility activity (Charcoal movement Test) of MELC.***

Treatment	Dose mg/kg(p.o)	% mean motility index± S.E
Control (2% v/v Tween 80)	**10ml/kg**	92.35±0.563
Loperamide	**4**	33.34±1.01*
Extract	**400**	58.26±0.615*a
Extract	**600**	47.91±0.620*a
Extract	**800**	27.83±1.09*a

*p value <0.01 vs. control, [a]p value< 0.01 vs. Loperamide

Each value represents the average for four mice. The Antimotility effect of MELC was analyzed by using ANOVA(Post hoc turkey method).The mean difference is significant at 0.0 level.

Table 4: ***Analgesic activity of different concentration of MELC***

Treatment	Dose mg/kg(p.o)	Reaction time in seconds (mean±SEM)			
		0 min	30min	60min	120min
Control	10ml/kg	2.40±0.10	2.40±0.03	2.33±0.08	2.35±0.05
Aspirin	300mg/kg	2.40±0.04	6.78±0.03[a]	7.49±0.04[a]	4.44±0.04[a]
Extract	400mg/kg	2.41±0.05	3.89±0.03[ab]	4.79±0.04[ab]	3.70±0.05[ab]
Extract	600mg/kg	2.41±0.07	4.37±0.04[ab]	5.89±0.03[ab]	4.97±0.06[ab]
Extract	800mg/kg	2.41±0.06	6.58±0.06[a]	6.83±0.03[ab]	4.55±0.04[a]

[a] $p<0.01$ vs. control, [b] $p<0.01$ vs. aspirin

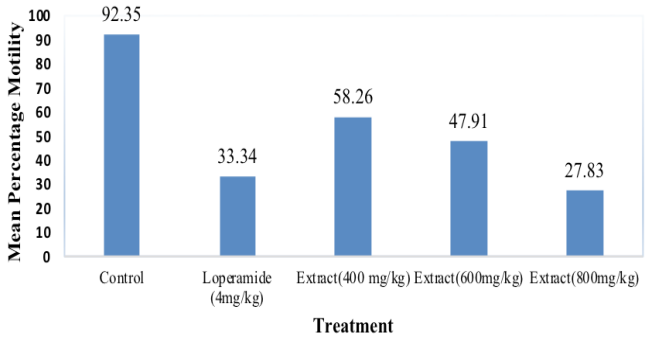

Figure 3: ***Antimotility effect of MELC by charcoal movement test.***

Antidiabetic activity: Transient hyperglycemia was produced by oral glucose tolerance test i.e. loading 3g/kg D-glucose after 30 minute of extract administration. Significant peak increase in blood glucose concentration was found after 30 minute of glucose loading and decreased gradually in all tested group. The hypoglycemic effect of MELC was found maximum at 60 min after glucose load. Hypoglycemic effect of Glibenclamide 5mg/kg, MELC 400mg/kg and MELC 800mg/kg at 60 min was found to be 71.32%, 46.58% and 38.38% as compared with the control. The MELC showed significant reduction of glycemia in dose dependent manner.

Values are mean ±SEM of four rat. The hypoglycemic effect of extract was analyzed by using one way ANOVA (post hoc Tukey method). The mean difference between the control, standard and different doses of extract was found to be significant ($p<0.05$). Also significant dose dependent activity of the plant extract has been found ($p<0.05$)

Table 7: ***Antihyperglycemic effect of different concentration of MELC and glibenclamide compared with control***

Sample	Dose mg/kg p.o	% antihyperglycemic effect		
		30min	60min	90min
Glibenclamide	5	50.28	71.32	74.47
Extract	400	14.37	38.38	17.95
Extract	800	37.11	46.58	44.1

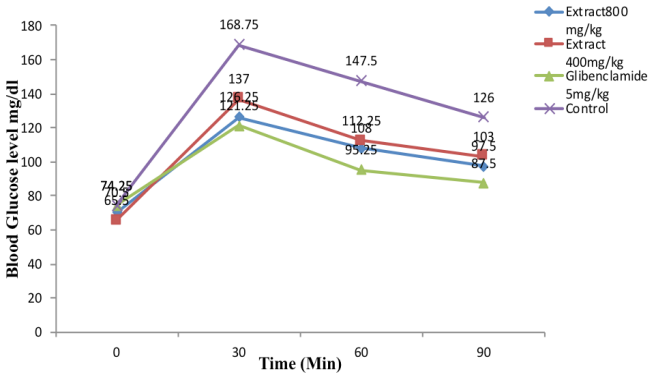

Figure 4: ***Reduction in blood glucose level by different doses of MELC and glibenclamide at various time intervals***

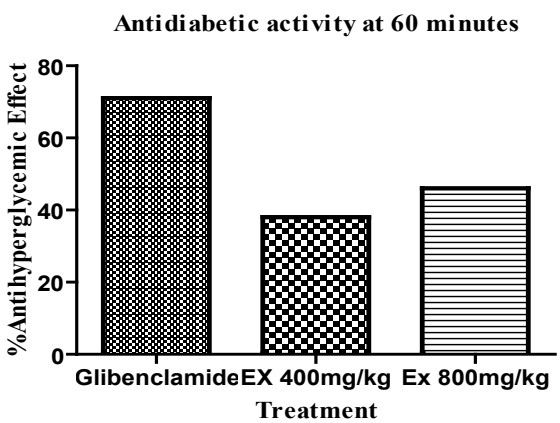

Figure 5: ***Antihyperglycemic effect of Glibenclamide and different doses of MELC at 60 minutes***

Table 6: Hypoglycemic activity of different concentration of MELC and Glibenclamide on glucose loaded rats.

S.N	Sample	Dose mg/kg p.o	Initial glucose level at 0 min	Glucose level mg/dl±SEM 30min	60min	90min
1	Control	10ml/kg	74.25 ±1.55	168.75±1.31	147.5±2.25	126± 1.29
2	Glibenclamide	5	74.25± 1.11	121.25± 1.49[a]	95.25± 1.11[a]	87.5 ±1.85[a]
3	Extract	400	65.5±0.65	137±1.29[ab]	112.25±1.31[ab]	103±2.12[ab]
4	Extract	800	70.75± 1.11	126.25±1.11[a]	108±1.08[ab]	97.5±1.19[ab]

[a]$p<0.05$ vs. control,[b] $p<0.05$ vs. glibenclamide

Discussion

This study was carried out to find the major groups of phytochemicals present and to evaluate the biological activities of the plant. The preliminary phytochemical screening of the MELC was found to exhibit the positive reaction tests for tannins, diterpenes, triterpenes, flavonoids, carbohydrates, saponins, steroids, glycosides where as petroleum ether extract showed positive tests for saponins, diterpenes, carbohydrates and steroids (Table 2). Similar kind of study was performed by Kalita et al by successive extraction of *L. camara* leaves extract by petroleum ether, methanol and water. MELC showed the presence of carbohydrates, tannins, saponins, flavonoids, glycosides and triterpenoids where as petroleum extract showed the presence of tannins, steroids and triterpenoids [44]. Thus phytochemical screening helped in identifying the main chemical constituents present in the extract.

The antimicrobial potential of MELC was carried out by well diffusion method using two types of bacteria: a gram positive *S. aureus* and a gram negative *E. coli.* The plant extract showed activity against *S. aureus* whereas did not show any zone of inhibition against *E.coli (*Table 3*)*. Similar kind of antimicrobial study performed by Ambiye *et al* used petroleum ether, chloroform, methanol and water extract of aerial parts of *L. camara* against 2 gram positive and 2 gram negative bacterial including *S. aureus* and *E. coli*. None of the extract showed the zone of inhibition against *E. coli* where all the extract showed significant zone of inhibition against *S. aureus,* which is consistent with my study [45]. Susceptibility differences between gram positive and gram negative may be because of structural differences in cell wall between class of bacteria [45]. Bioactive triterpine-22 beta-acetoxylentic acid and other triterpine showed the antimicrobial activity against this extract [46].

Despite its wide spread medicinal use, *L. camara* is listed as one of the poisonous plant for grazing animal such as cattle and sheep [47]. But different studies indicated that intoxication only occurs when plant material (>1% of the body weight) is ingested. The acute toxicity done on present study showed that the methanolic extract of the plant had LD_{50} >2000mg/kg. Similarly other study done by Poue M et al on the leaves and Tadesse et al on stem of *L. camara* plant showed the same results i.e. LD_{50}>2000 mg/kg [48-49].

The analgesic activity of the MELC was determined by tail immersion method. The different doses of extract showed significant analgesic activity in dose dependent manner (Table 4). In the similar experiment done by using *L. camara* plant extract showed the significant antinoceptive action at 1g/kg i.p respectively [50].

The significant increase in pain threshold as indicated by a rise in reaction time or maximum possible analgesia of the extract and standard drugs of tail immersion method suggest the involvement of central pain pathway i.e. centrally mediated anti-nociceptive action [51-52]. However, to confirm the involvement of opioid receptor in mechanism of analgesia tail immersion method need to be repeated by co-administering extracts and Naloxone (non-selective opioid receptor antagonist) [53]. However further study is warranted to identify the active compounds present in the extract and to elucidate the actual mechanism involved in the analgesia.

The antimotility test was carried out by charcoal movement test. There was significant difference in the antimotility activity between different concentration of MELC i.e. they exhibit dose dependent antimotility effect (Table 5). High dose 800mg/kg was found to be more potent than loperamide 4mg/kg. Neostigmine induced gastrointestinal motility activity of MELC was performed by Ojha et al which showed the potent antimotility effect i.e. 1g/kg completely inhibited the intestinal transit [54]. Protein tannates produced by tannin by denaturation of protein make intestinal mucosa more resistant and reduce secretion [55]. Flavonoid and phenol cause antidiarrhoeal activity due to their antioxidants and free radical scavenging activity [56]. Therefore the antidiarrhoeal activity of *L. camara* leaf extract may be due to flavonoid and tannin present in it.

The antidiabetic activity of the MELC was carried out by oral glucose tolerance test by loading 3 g/kg D-glucose MELC showed the significant decrease in

blood glucose concentration in dose dependent manner (Table 6-7). The antidiabetic activity of *L. camara* performed on alloxan induced diabetic rat showed the significant decrease in blood glucose concentration in dose dependent manner. Several researcher reported that flavonoids, steroids, terpenoids, saponin are known to be bioactive antidiabetic principle [57-58]. Flavonoids regenerate the damaged cells and act as insulin secretagogues [59]. Saponin reduces uptake of certain nutrients such as glucose and cholesterol at the gut through intraluminal physiochemical reaction [60]. Glibenclamide acts by interacting with sulfonyl urea receptors on the beta cells and by interfering with ATP sensitive potassium channels on pancreatic beta cells, which increase the secretion of insulin. It also increases the sensitivity of existing insulin receptors [61]. Thus, the antidiabetic effect of *L.camara* extract may be due to enhancement of insulin secretion or reducing the uptake of glucose from gut.

As this plant showed antimicrobial, analgesic, antimotility, antidiabetic activities, detailed phytochemical studies and other research works are needed to confirm the identity of the bioactive principles responsible for these actions.

Conclusion and recommendation

The present study has opened opportunities for further research especially regarding development of potent formulation for diabetes mellitus and diarrhoea from *L. camara* leaves. Therefore from this study, it could be concluded that MELC possessed medicinal values which could justify scientifically the ethno-medical importance and help to generate safe and effective pharmaceutical ingredients.

Detailed phytochemical studies should be done to isolate, purify and identify pharmacologically active principle responsible for biological activities. Hence, further scientific investigation and specific studies are highly recommended for better evaluation of the potential effectiveness of the plant, so that it can be utilized as an alternative in future as well as ethno-medical knowledge about such plants within the region should be documented along with scientific evidences.

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